nsls
IR UV X

Science Highlights

from the National Synchrotron Light Source

BEAMLINE
X19C

PUBLICATION
Ming Li, David J. Chaiko, and Mark L. Schlossman, "An X-ray Reflectivity Study of a Monolayer of Ferritin Proteins at a Nano-film Aqueous-Aqueous Interface", *J. of Phys. Chem. B,* **107**, 9079-9085 (2003).

FUNDING
National Science Foundation Division of Materials Research; Petroleum Research Foundation administered by the American Chemical Society

FOR MORE INFORMATION
Mark Schlossman, Department of Physics, U. of Illinois at Chicago
schloss@uic.edu

A Monolayer of Ferritin Proteins at a Nanofilm Aqueous-Aqueous Interface

Ming Li[1], David J. Chaiko[2] and Mark. L. Schlossman[3]

[1]Department of Physics, University of Illinois, Chicago, IL; [2]Argonne National Laboratory, Argonne, IL; [3]Departments of Physics and Chemistry, University of Illinois, Chicago, IL

The formation of thin aqueous films on top of an aqueous subphase is demonstrated. The films form via a complex spreading process that results in the coexistence of macroscopic lenses and films that are several nanometers thick. Biomolecules, such as proteins, can be trapped at the aqueous-aqueous interface or in the thin film. This idea is demonstrated by an x-ray reflectivity study of ferritin proteins that form a two-dimensional assembly at the interface. It is shown that the electron density interfacial profile of the ferritin trapped in this thin film is consistent with the known crystal structure of ferritin.

Although interfaces between bulk liquids are commonly formed between immiscible liquids, such as oil and water, it is also possible to form an interface between two aqueous solutions. For example, aqueous mixtures of polymers, salts, and water are known to separate into two or more equilibrium phases. Aqueous biphase systems (aqueous mixtures that form two phases) are used for the purification and separation of biological materials. This is possible because, although biological materials are soluble in both aqueous phases, they prefer to be in one or the other, and are therefore separated into one of the two. We have chosen to use mixtures of polyethylene glycol (PEG), potassium phosphates, and water to investigate the formation of protein assemblies in layers of aqueous solutions that are approximately four nanometers thick. Both the PEG and the potassium phosphates are compatible with many proteins, making the mixture an appropriate choice.

We have shown that thin layers of aqueous solutions supported on a bulk aqueous subphase can be formed from biphase mixtures by the following procedure: Upon mixing the PEG, salt, and water, the resulting bulk liquid separates into a lower salt-rich phase and an upper PEG-rich phase. After extracting the salt-rich phase into a separate container, a single drop of the PEG-rich phase is placed onto the surface of the salt-rich phase. The drop spreads across the entire surface, thins rapidly, and then breaks up into smaller drops that coarsen to form flat lenses with diameters on the order of one centimeter (**Figure 1**). Then, using a pipette, any lenses in the path of the x-ray beam are either aspirated off the surface or moved off to the side (at least one lens remains on the surface during the x-ray measurements). These macroscopic lenses function as reservoirs to supply the PEG-rich thin film.

Mark L. Schlossman

We used x-ray reflectivity to demonstrate that a four nanometer-thick film exists in the region between the macroscopic lenses. This region is a thin layer of the bulk PEG-rich phase, rather than a monolayer of PEG. Since proteins retain their natural conformation in PEG solutions and protein dimensions are typically similar to the film thickness, it is natural to ask if this thin film can be used to collect proteins into two-dimensional assemblies.

In an experiment to test the proof of this principle, we demonstrated that ferritin proteins can be assembled into the PEG-rich thin film. Ferritin is used for the storage of iron, and consists of a nearly spherical organic shell surrounding a nearly spherical core in which the iron is stored. The ferritin is prepared in a solution of water and PEG, whose composition mimics that of the PEG-rich solution in the biphase system. A few drops of the ferritin solution are added to the lower salt-rich bulk phase. Then, they rise to spread into the PEG-rich thin film. **Figure 2** illustrates the electron density profile of this film, as measured using x-ray reflectivity, and compares it to the predicted profile of a layer of ferritin calculated from the known

molecular structure of the protein. The near-match of measured and calculated profiles indicates that the ferritin forms a two-dimensional film that is ordered normal to the interface.

The overall method described here allows proteins or other biomolecules to be assembled two-dimensionally and studied with x-rays. This is significant because many proteins do not crystallize in three dimensions, or may be difficult to crystallize and, therefore, cannot be studied that way. Allowing proteins to assemble at an aqueous surface often results in protein denaturation, causing the loss of the protein's biological activity. Our method retains the natural shape of the protein at a liquid surface. Moreover, the formation of these two-dimensional arrays may be useful technologically because the arrays allow the biomolecules to interact with other molecules. This could be utilized in chemical sensing or catalytic applications.

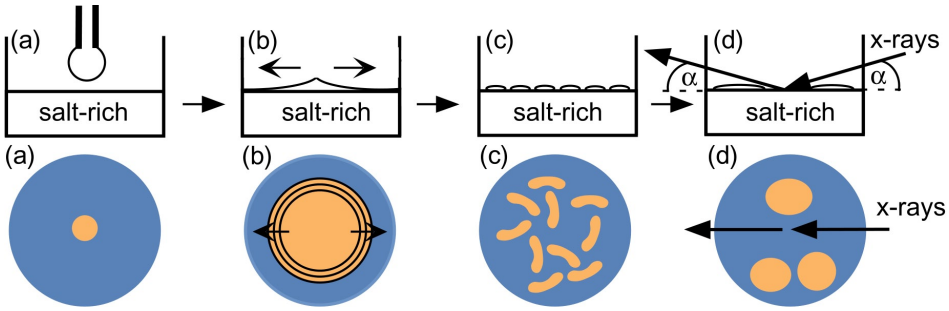

Figure 1. *(a) Thin layer is formed by placing a drop of the PEG-rich phase on the surface of the salt-rich phase with a pipette (top illustrations are a side view, bottom are a top view). (b) Over a period of about one second the drop spreads and a ring of interference colors can be observed. (c) After about 10 seconds, small islands form a spinodal-like pattern (not as ordered as the illustration). (d) The pattern coarsens over a period of about one hour, leaving a few large (approximately one centimeter in diameter) lenses. The lenses are pushed aside with a pipette to allow access for the x-rays that probe the region between the lenses. This region consists of a four nanometer-thick film of a PEG-rich solution.*

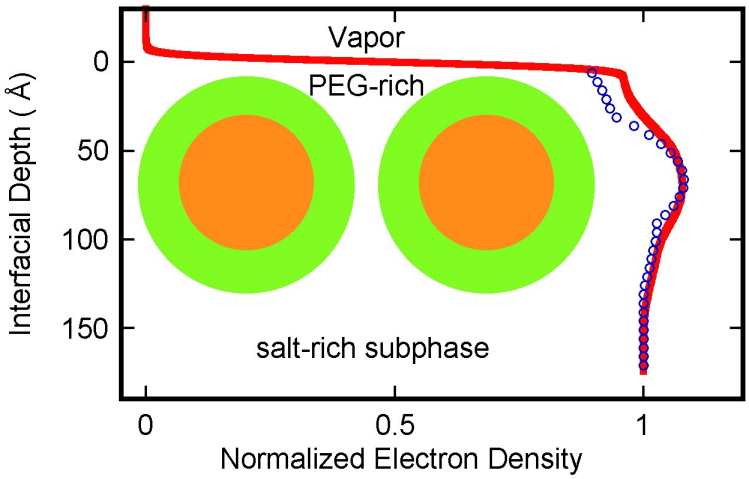

Figure 2. *The electron density profile of ferritin adsorbed at the interface formed by the PEG-rich thin film and the salt-rich subphase. The solid curve is the profile determined by x-ray reflectivity, and the small circles are a calculation of the profile assuming a single layer of ferritin proteins. The illustration represents (nearly to scale) the position of the ferritin layer used for the calculation. The colored circular regions represent the core and shell of the ferritin.*